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Note

Rapid gas chromatographic method for the determination of kepone in the eel

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Kepone (chlordecone; 1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[*cd*]pentalen-2-one) is used as an insecticide, larvicide and fungicide and is a toxic, cumulative compound. In nature, kepone is formed as a degradation product of the insecticide kelevan, used in potato culture[1].

A kepone pollution incident in the James River (U.S.A.) and the import into The Netherlands of possibly contaminated eels from this area were the reason for this investigation.

Previously described procedures for the determination of kepone used several solvent systems and several clean-up methods[2–4]. The preferred procedure used by Moseman *et al.*[5] for the determination of kepone in fish and shellfish was homogenization with toluene–ethyl acetate, clean-up of the extract by gel permeation chromatography and subsequent elution through a Florisil column.

Fish may contain other organochlorine pesticides and polychlorobiphenyls. An efficient method for the separation of the organochlorine pesticides from the polychlorobiphenyls with aluminium oxide column and activated silica gel columns is used in many laboratories in The Netherlands[6–8].

This paper presents a more rapid method than those described in the literature.

EXPERIMENTAL

Chemicals and reagents

Silica gel (Merck, Darmstadt, G.F.R.; No. 7754, 60 reinst, 70–230 mesh) was activated at 200° for about 2 h, then cooled to room temperature in a desiccator. The silica gel was deactivated with water as follows. Water was distributed on the inside of a glass-stoppered bottle, activated silica gel was added in an amount to make the portions of silica gel and water 97:3 (w/w) and the bottle was shaken for several minutes. The silica gel could be used after 1 h, with occasional shaking prior to use.

For column chromatography, glass columns (500 × 6 mm I.D.) were used, to which quartz-wool, 0.5 g anhydrous sodium sulphate, 2 g of silica gel freshly prepared as described above and 0.5 g anhydrous sodium sulphate were added successively. Before use, the column was washed with 5 ml of dichloromethane–*n*-hexane (1:1, v/v).

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Kepone (Allied Chemicals) (analytical-reagent grade) was dissolved in acetone–*n*-hexane (5:95); dilution with *n*-hexane yielded standard solutions containing 0.1–1.0 ng of kepone per 5 μl.

Gas chromatograph

A Hewlett-Packard HP 5710A gas chromatograph with a nickel-63 detector was used. A Pyrex column (1750 × 2.7 mm I.D.) was packed with Chromosorb W AW DMCS (80–100 mesh), coated with 10% OV-210 and 10% OV-17 (4:1). Argon–methane (95:5) was used as the carrier gas at a flow-rate of 60 ml/min. The column, detector and injection port temperatures were 200°, 250° and 210°, respectively.

The retention time of kepone relative to aldrin was 2.75. The sensitivity was 0.7 ng of kepone per half-scale deflection.

Extraction

A 20-g amount of chopped eel and 20 g of anhydrous sodium sulphate were macerated in 100 ml of isopropanol–chloroform (1:1) by means of an Ultraturrax for about 2 min. After equilibration for 16 h the mixture was macerated for a further 2 min, then centrifuged for 5 min at 2500 *g* and the extract was collected.

Clean-up

A 50-ml volume of the extract, corresponding to 10 g of eel, was evaporated to dryness by means of a rotary vacuum evaporator. The residue was dissolved in 50 ml of *n*-hexane and extracted three times with 40 ml of acetonitrile (saturated with *n*-hexane) within 2 min. The collected acetonitrile layers were washed with 50 ml of *n*-hexane and evaporated to dryness.

The residue was transferred quantitatively to a silica gel column, using about 2 ml of dichloromethane–*n*-hexane (1:1). The column was eluted with 50 ml of dichloromethane–*n*-hexane (1:1) and the eluate (eluate I) was discarded. The column was then eluted with 150 ml of dichloromethane–*n*-hexane (1:1), the eluate (eluate II) was evaporated to dryness and the residue was dissolved in 5 ml of isopropanol.

Gas chromatography

A 5-μl volume of the solution was injected into the gas chromatograph. Standard solutions containing 0.1–1.0 ng of kepone per 5 μl were also injected.

RESULTS AND DISCUSSION

Eluate I contained organochlorine pesticides and polychlorobiphenyls, but partitioning with acetonitrile did not extract the organochlorine pesticides completely. For the quantitative determination of these components, the first macerate must be used.

Kepone was determined in eluate II. The limit of detection was 0.005 ppm of kepone based on the weight of fresh eel taken.

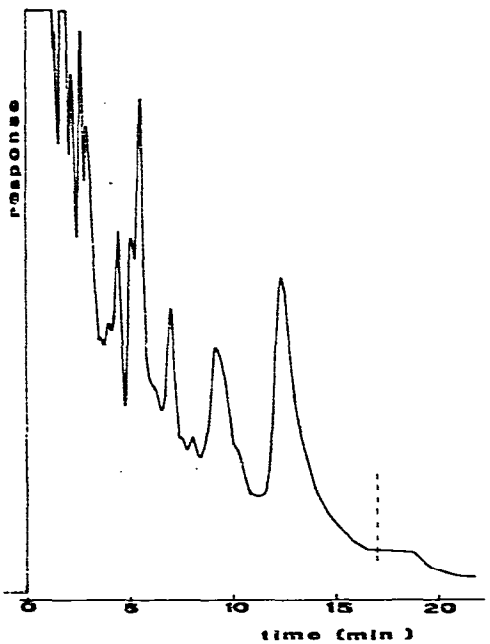
Experiments in which eel (containing 0.14 ppm of kepone) was extracted in various ways showed that extraction with isopropanol–benzene (1:1) or isopropanol–toluene (1:1) was as efficient as extraction with isopropanol-chloroform (1:1). However, maceration with *n*-hexane or Soxhlet extraction with *n*-hexane yielded only 25

TABLE I

RECOVERIES OF KEPONE FROM BLANK EEL TREATED WITH KEPONE STANDARD SOLUTIONS

Average values are given for five recovery experiments, each carried out with five concentrations of kepone. The standard deviation was 12%.

Kepone added (fresh eel basis) (ppm)	*Average recovery (%)*
0.011	82
0.055	88
0.111	86
1.11	94
5.56	101



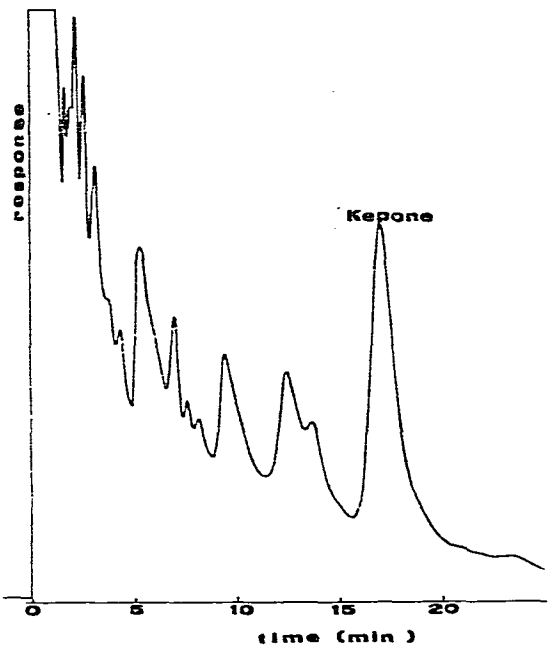


Fig. 1. Chromatogram of eluate II from blank eel. The broken line indicates the position where the kepone peak would be expected.

Fig. 2. Chromatogram of eluate II from eel containing 0.07 ppm of kepone.

and 50% of the kepone, respectively, compared with the amounts obtained with our suggested extraction procedure.

In recovery experiments in which standard solutions of kepone were added to the macerate, high recoveries were obtained (Table I).

The method was tested on 48 samples of eel (fresh, frozen or smoked). When present, kepone could be determined without interferences from other compounds (Figs. 1 and 2). Positive samples were identified by gas chromatography–mass spectrometry (*m/e* 486, 270, 235, 216).

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